Appendix A : CHANGES TO SPECIFICATION, PAGES 1 AND 7

HUMAN GLUCOCORTICOID RECEPTOR 1A PROMOTER AND SPLICE VARIANTS

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This invention pertains to the location of a human glucocorticoid receptor gene promoter region and to three splice variants of the human glucocorticoid receptor gene; and the use of the promoter region and of the splice variants to improve the diagnosis and treatment of leukemia.

The use of naturally occurring substances, such as hormones, to treat cancer has certain advantages. Although side effects can occur, the effects are usually less severe than those caused by cytotoxic chemotherapy. Unfortunately most cancers are not effectively controlled by hormonal therapy, but exceptions include certain hormonally-dependent breast cancers that can be treated with the anti-estrogen tamoxifen, and acute promyelocytic leukemia that is responsive to all-*trans* retinoic acid. Additionally, some lymphoid malignancies can be effectively treated with glucocorticoid steroid hormones, hormones that control a variety of metabolic and developmental processes. See R. R. Denton *et al.*, "Differential Autoregulation of Glucocorticoid Receptor Expression in Human T- and B-Cell Lines," *Endocrinology*, vol. 133, pp. 248-256 (1993). Certain types of B- and T-cell acute lymphoblastic leukemia ("ALL") are particularly sensitive to glucocorticoid hormonal therapy. Glucocorticoids affect lymphoid malignancies due to the induction of programmed cell death, or apoptosis, of immature

based upon the absence of these sequences in mRNA. There are ~981 bp of exon 1A sequence. The portions of the hGR 1Ap/e sequence that can function as a eukaryotic promoter or as intraexonic regions that influence promoter activity were identified based on reporter gene assays. The detection of exon 1A3-containing transcripts can be used for the diagnosis of patients with T-cell acute lymphoblastic leukemia (ALL) and other glucocorticoid-responsive cancers, and to identify patients who would benefit from glucocorticoid hormone treatment.

Brief Description of the Drawings

Fig. 1-1 through 1-2 documents the DNA sequence of the hGR 1Ap/e region, SEQ ID NO: 1. The putative transcription start (CAP) site is denoted as base 1. The boxed series of bases indicate regions protected using DNAse I footprinting. The primer sequences are indicated by dashed arrows under the sequence. The vertical arrows illustrate splice donor sites. The italicized sequence indicates the portion of the sequence of which the complimentary strand is a portion of GenBank Accession # AA917693 (with two mismatches).

Fig. 2-1 through 2-2 illustrates the alignment of the hGR 1Ap/e promoter sequence, SEQ ID NO: 2, the top sequence, with the mouse 1A promoter sequence, SEQ ID NO: 3, (Chen *et al.*, 1999b) using the ALIGN program.

Fig. 3-1 through 3-2 illustrates the alignment of the hGR 1Ap/e exon sequence, SEQ ID NO: 4, the top sequence, with the mouse 1A exon sequence, SEQ ID NO: 5, (Chen *et al.*, 1999b) using the ALIGN program.

Fig. 4 illustrates a schematic diagram of the genomic structure of the human GR (hGR) promoters and the GR gene up to exon 2.

Fig. 5 illustrates the relationships of the three exon 1A transcripts to the hGR 1A promoter and the GR gene up to exon 2.

Fig. 6 illustrates the expression of hGR exon 1A3, 1B, and 1C transcripts in various cell lines and adult brain tissue.

Fig. 7 illustrates the expression of hGR exon 1A1, 1A2, and 1A3 transcripts in various cell lines and adult brain tissue.

Fig. 8 illustrates the effects of glucocorticoid treatment on expression of GR exon 1 transcripts in two leukemia cell lines.

Fig. 9 illustrates the effects of glucocorticoid treatment on cells transfected with a portion of